REMARKS

Claims 1 – 3, 6 – 9, 11, 12 and 18 are pending in the application. Claims 5, 6, 10 and 13-17 have been previously cancelled. Claim 18 has been amended. No new claims have been added. No new matter has been added.

Any cancellation of the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

Claim Rejections Withdrawn

The Examiner has withdrawn the rejection to claims 1 – 3, 6 – 9, 11 – 12 and 18 under 35 USC §112, second paragraph.

Claim Rejections 35 USC §103(a)

Claims 1 – 3, 6 – 9, 11 – 12 and 18 have been rejected under 35 USC §103(a)as being unpatentable over the combination of Marcato et al. (Infection and Immunity vol. 70 p.1279 (2002) in view of LaCasse et al.(Blood vol. 88 p.1561 (1995)) and Strockbine et al. (J Bacteriology vol. 170 p.1116), Accession Number 2002:397002, Green (US 2002/0081307) and Applicant's admission on page 6, lines 1 – 2 of the specification. (Office Action, p.3). Applicants respectfully disagree.

The present claims recite a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a Stx1B subunit of Shiga toxin.

As discussed in the previous response, Applicants have **particularly identified Stx1B for use in the methods as claimed**. Applicants teach that there are a number

of Shiga toxin variants and subunits, for example at page 6, beginning at line 30 of the present disclosure:

> The sequences of numerous Shiga toxin variants and subunits are known in the art. For example, the Shiga toxin 1 B-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession Nos. 32400300 and 32400303, the Shiga toxin 2 B-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession No. 13359150, the Shiga toxin 1 A-subunit is set from the E. coli O157:H7 strain is set forth in GenBank Accession Nos. 32400299 and 32400302, and the Shiga toxin 2 A-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession No.15718405.

Among all of these variants and subunits, **Applicants have particularly identified Stx1B for use in the methods as claimed**.

Further, Applicants point out that in the present work, recombinant Stx1B has been used to demonstrate the effects of reducing, or inhibiting invasiveness and metastasis of tumor cells, as claimed. In Applicant's work, recombinant B subunit of Shiga toxin (Stx1B) was obtained from the GRASP Center, New England Medical Center (see, e.g. p. 36, line 4-5). Applicants show that the recombinant B-subunit alone causes apoptosis in human colon cancer cells (see, e.g. Example 7 at p. 43). In Example 8, on pages 43 - 44, Applicants show that Stx1B selectively causes apoptotic death in cells expressing Gb3. It is well known to one of skill in the art that the Stx holotoxin has very different effects from the individual subunits. It is well known to one of skill in the art that the holotoxin contains both the enzymatic Stx A and five Stx B subunits, and that the A subunit is well-known to be toxic.

None of the references cited by the Examiner, taken alone or in combination, teaches or suggests the present invention as claimed.

The test of obviousness requires that one compare the claimed "subject matter as a whole" with the prior art "to which said subject matter pertains" 35 U.S.C. § 103(a). To establish a prima facie case of obviousness, three criteria must be met. First, a suggestion or motivation to modify the reference or combine reference teachings must be present in the references or in the general knowledge present in the art. Second, there must be a reasonable expectation of success. Finally, the prior art reference must

teach or suggest all the claim limitations. M.P.E.P. 2143. The burden is on the Examiner to show that the references expressly or impliedly suggest all of the claim limitations. M.P.E.P. 2142. "There are three possible sources for a motivation to combine references: the nature of the problem to be solved, the teachings of the prior art, and the knowledge of persons skilled in the art." In re Rouffet, 149 F.3d 1350, 1357. In the absence of some teaching or suggestion to combine, no prima facie case of obviousness can be established, and the rejection is improper and must be withdrawn. In re Fine, 837 F.2d 1071, 1074.

In the present case, the references cited by the Examiner fail to provide the requisite motivation to combine, fail to provide a reasonable expectation of success, and fail to teach or suggest all of the claim limitations. Each of the references cited by the Examiner in support of the obviousness rejection is considered below.

The Marcato et al. reference is directed to the use of the cloned shiga toxin B (Stx2 B) subunit to induce apoptosis in Burkitt Lymphoma B-cells. Applicants attach a Retraction of this work by Mercato (Mercato et al., Infection and Immunity, Aug. 2003, p.4828; provided herein), where Mercato et al. indicate that

> we discovered that additional preparations of cloned Shiga toxin 2 (Stx2) b subunit lacked apoptogenic activity in Ramos Burkitt's lymphoma B cells…We discovered that the Stx B subunit preparations used in our study contained previously undetected Stx2 holotoxin. **Since this contaminating Stx2 holotoxin was likely responsible for the apoptogenic activity we attributed to the Stx2 B subunit** in this article, we retract the conclusion that the Stx2 B subunit, absent any subunit activity, initiated apoptosis in Ramos cells. **This new finding does not alter our conclusions related to the lack of apoptogenic activity in the Stx1 B subunit preparation**…

As indicated above by Mercato et al., the Examiner's argument on p. 6 of the Office action, that "Applicant's misplaced assertion that apoptosis was not observed in the A subunit free preparations of the Stx1 B pentamer" is incorrect. **The Stx 1B**

subunit does not show apoptotic activity. As described by Mercato et al., above, there is clearly a difference in activities between the holotoxin and recombinant Stx subunits. As discussed, the holotoxin contains both the enzymatic Stx A and five Stx B subunits, and, as shown by Mercato et al. above, the A subunit is well-known to one of skill in the art to be toxic.

Accordingly, the teachings of Marcato would not lead one of skill in the art to choose StxB1 as an apoptosis inhibitor in the claimed methods.

Nowhere in the Marcato reference is there teaching or suggestion of a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a Stx1B subunit of Shiga toxin as claimed.

None of the LaCasse, Strockbine or Greene references cure the defects of the Marcato reference. None of the references, alone or in combination, teach or suggest a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a Stx1B -subunit of Shiga toxin.

As previously discussed, the LaCasse reference is directed to the use of shiga like toxin (SLT-1) in human bone marrow (BM) purging. LaCasse uses Shiga Like Toxin (SLT-1) which kills cells by inhibiting protein synthesis. (p.1561). The purpose of the study described by LaCasse "was to establish the potential of a natural toxin (SLT-1) in purging B-cell lymphomas from BM." (p.1563).

LaCasse does not teach or suggest a method of reducing or inhibiting invasiveness and metastasis of tumor cells in a subject using Stx1B.

The Examiner argues that "LaCasse et al disclose treatment of human B cell lymphoma from bone marrow in mice using Shiga-like toxin 1 (and) also discloses that the toxin was administered after the cancer is present." (Office Action, p.4). The Examiner argues further that "(o)n page 6 of the specification, Applicant admits the toxins are known to bind to Gb3 expressing cells, therefore it is expected that the cells of the reference are Gb3 expressing cells." (Office Action, p.4).

Further, Applicants point out that LaCasse purifies the B-subunit of SLT-1 from an E.coli culture as described by Ramotar et al. (Biochem J. 1990. 272. 805-811; attached). It was well-known to one of skill in the art at the time of filing that *E.coli* was not a reliable system for producing the B-subunit. Referring to p. 1990 of the Ramotar reference, Ramotar teach that "(i)n five separate experiments, the first two polymixin B extracts yielded SLT-1B at quantities of 80+/-58 and 48+/-25 µg/ml of the original culture, respectively…(t)he mean total yield from polymixin B extracts and pellet wash was 160+/-79 µg/ml of culture." These are terrible yields. Moreover, this result demonstrates that 50% in total yield of Stx1 B was unknown/ uncharacterized substances, which can produce many unknown side effects in cancer models, including apoptosis. Further, Figure 2, lane D, shows that there was no detection of the purified B-subunit of Stx-1. Given these results, it is not clear what LaCasse et al. were actually using in their experiments. It was known in the art at the time of filing that *Vibrio cholera* was the organism of choice for producing shiga toxin. Applicants attach the Acheson reference (Infection and Immunity, Jan. 1996, p.355 – 357; provided herein), that teaches, using *V. cholera*, large amounts of recombinant SLT-1B subunit can be produced in vitro, for example, over 10 mg of SLT-1B have been purified per liter of culture. (p.356).

It would not have been obvious to one of ordinary skill in the art that StxB subunit can also be used to inhibit apoptosis in vivo, as argued by the Examiner on page 5 of the Office Action. Thus, the teachings of Marcato would not motivate one to use Stx1B in place of SLT-1, as taught by LaCasse. The teachings of the cited art, when combined, do not result in the claimed invention.

Accordingly, Applicants request that the rejection be withdrawn and the claims allowed.

CONCLUSION

In view of the above amendment, applicant believes the pending application is in condition for allowance.

Dated: September 9, 2010

Respectfully submitted,
Electronic signature: /Peter F. Corless/

Peter F. Corless
Registration No.: 33,860
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5557
Attorneys/Agents For Applicant